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Research Article

INSIGHTS INTO THE ROLE OF *MORUS ALBA* IN REVERSING OBESITY-ASSOCIATED HEPATIC STEATOSIS AND RELATED METABOLIC DISORDER IN RATS

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ABSTRACT

Objective: The goal of the present study was to examine the viability of *Morus alba* (*M. alba*) ethanolic extract in repression of obesity-associated hepatic steatosis and related metabolic disorder; dyslipidemia, hyperinsulinemia, and glycemic status.

Methods: Adult female albino rats were randomly assigned into four groups, eight rats each as follows: Group (1) control group received standard rodent diet for 24 weeks. The other three groups administered high cholesterol diet for 12 weeks and served as obese group, *M. alba*-treated group, and simvastatin-treated group.

Results: The current results showed an increment in thoracic circumference (TCX) and abdominal circumferences (AC) as well as body mass index (BMI) in obese group. In addition, dyslipidemia, hyperinsulinemia, hyperglycemia, and insulin resistance have been elucidated in obese group. Moreover, hepatic malondialdehyde (MDA), nitric oxide (NO), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin values were significantly increased in obese groups versus control group. On the other hand, administration of ethanolic extract of *Morus alba* or simvastatin could significantly lessen BMI and in addition to improve dyslipidemia in obese group. Glucose, insulin levels, and insulin resistance value in serum samples demonstrated a significant reduction in obese group upon treatment with *M. alba* ethanolic extract or simvastatin. Furthermore, noticeable depletion in hepatic MDA, NO contents, serum ALT, AST activities, and serum bilirubin level was recorded as a result of treatment with either ethanolic extract of *M. alba* or simvastatin. Histopathological examination of liver tissue showed ballooning degeneration in the hepatocytes (hepatic steatosis) associated with inflammatory cells penetration in portal zone in obese group. Meanwhile, the treatment of obese groups with ethanolic extract of *M. alba* or simvastatin was found to restore the structural organization of the liver.

Conclusion: The present findings provide a novel aspect for understanding of the role of *M. alba* against obesity-associated liver diseases and related metabolic disorder. The mechanisms underlying these effects seem to depend on the hypolipidemic potential, anti-inflammatory property, and antioxidant activity of its phytochemicals.

Keywords: Obesity, *Morus alba,* Dyslipidemia, Hyperinsulinemia, Hyperglycemia, Hepatic steatosis.

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INTRODUCTION

Liver is one of the largest organs in the human body that regulates homeostasis of the body [1], metabolic [2], and excretory functions [3]. It involves almost all the biochemical pathways of growth, fight against disease, nutrient supply, and reproduction [4].

Obesity is a complicated multifactorial metabolic disorder induced due to an imbalance between energy intake and expenditure that may have genetic and/or behavioral causes including the amount and nature of food intake and way of life [5]. Obesity is identified by boosted body weight and abnormal expansion of fat tissue with undue fat deposition, associated with severe deregulation of the endocrine capacity of fatty tissue [6]. It is a condition linked with a bunch of chronic and progressive diseases that have various aspects of metabolic disorder including diabetes, hyperinsulinemia and insulin resistance, dyslipidemia, cardiovascular defects, hepatic and renal pathologies, inflammation, and cancer [7,8].

Hepatic steatosis or fatty liver is mainly due to obesity and metabolic disorder. Hepatic steatosis is recognized by infiltrated fats and accumulated lipids, especially triglycerides in the liver, associated with enhanced liver/body weight proportion [9].

Sustainable agents from natural sources could act as applicable alternatives to currently used synthetic drugs in the treatment of obesity-related disorders. This may be attributed to the toxic adverse effects of these synthetic drugs and their expensive prices which make them not easily attainable to many patients in developing countries like Egypt. Phytochemicals in most of the medicinal plants could protect against free radicals formation, degenerative disorders, and lifestyle-related diseases. *Morus alba* (Moraceae) has been reported to have hypolipidemic effects in diabetic patients and hyperlipidemic rodents. *M. alba* has been found to possess a unique nutritional profile containing proteins, phenolics, flavonoids, and anthocyanins that enhance its effect as natural potent tonic [10]. The active ingredients of *M. alba* extract particularly quercetin, rutin, and isoquercitrin capture free radicals revealing efficient antioxidant effect. Presence of prenylated flavonoids further confirms its antioxidant claims [11].

The current study was tailored to investigate the effectiveness of ethanolic extract of *M. alba* on obesity-related hepatic steatosis and associated metabolic disorder; dyslipidemia, hyperinsulinemia, and insulin resistance in a rat model of obesity.

METHODS

Materials

Chemicals and drugs

Cholesterol was purchased from Sigma Chemical Co., USA. Simvastatin was purchased from Commercial Market, Cairo, Egypt. It is manufactured by MSD B.V Co., UAE. All other reagents, solvents, and

chemicals used for analysis met the quality criteria in accordance with the International Standards.

Plant preparation and extraction procedures

Air-dried aerial parts of *M. alba* Lam. were purchased from a local market in Cairo, Egypt, and the plant was identified by a botanist of the herbarium at the Botany Department, Faculty of Science, Cairo University, Giza, Egypt.

Dried aerial parts of *M. alba* (2 kg) were pulverized into fine powder using a stainless steel blender and passed through a mesh opening of 35 mm sieve. Then, the powder was extracted by cold percolation with 95% ethanol (3 × 4 L) till exhaustion. Afterward, the ethanol extract was concentrated under reduced pressure to give 250 g of brown residue. The residue was kept in a refrigerator till used in the biological assay.

Biological assay

Animals and treatments

All experiments involving animals and tissue samples were conducted in accordance with the principles and guidelines for the care and use of laboratory animals in the National Institute of Health, USA. This study was approved by the Ethical Committee for Animal Experimentation, National Research Centre, Egypt.

A total of 32 adult female albino rats of Wistar strain weighing 130±10 g at 90 days of age were enrolled in the present study. The animals were obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were housed throughout the experiment (8 rats/cage) in polypropylene cages under specific pathogen-free conditions with controlled illumination (12 hrs light/12 hrs dark cycle), relative humidity (30-50%), and temperature (18-22°C). Animals were fed with standard laboratory rat diet and water provided *ad libitum*. Animals were allowed to adapt to their environment for 2 weeks before the commencement of the experiment.

After the acclimatization period, eight rats were fed with standard laboratory rat diet containing 26.5% protein, 3.8% fat, 40% carbohydrate, and 4.5% crude fiber in 100 g of chow during 24 weeks of the experimental period and served as incline control group. The other 24 rats were fed with high-cholesterol diet (HCD) containing 19.93% protein, 15% cholesterol, 57.50% carbohydrate, and 2.81% dietary fiber in 100 g of chow (modified method of Soliman *et al*. [12]) for 12 weeks. The dietary ingredients were homogenized in distilled water and dried in an incubator at 60°C for 24 hrs and cut into small equal-sized pieces (pellets). HCD was given fresh each day as dry pellets; therefore, there was no spillage [13]. These rats were further assigned into three groups: Obese group in which the rats were fed with HCD for 12 weeks, then fed with standard laboratory rat diet for other 12 weeks; *M. alba*-treated group in which the rats were fed with HCD for 12 weeks, then fed with standard laboratory rat diet with simultaneous administration of *M. alba* ethanolic extract by intragastric gavage tube in a dose of 250 mg/kg b.wt. according to Sarikaphuti *et al*. [14] for 12 weeks; and simvastatin-treated group in which the rats were fed with HCD for 12 weeks, then fed with standard laboratory rat diet with simultaneous administration of antihypercholesterolemic drug (simvastatin) by intragastric gavage tube in a daily dose of 5 mg/kg b.wt. according to Mbikay [15] for 12 weeks.

Methods

Anthropometrical measurements

After animal treatment was over, rats in the different studied groups were fasted overnight (12-14 hrs), and the abdominal circumference (AC) (immediately anterior to the forefoot), thoracic circumference (TC) (immediately behind the foreleg), body length (nose-to-anus or nose-anus length), and body weight were measured following light ether anesthesia. Body length and body weight were used to determine body mass index (BMI) [13].

$$\text{BMI (g/cm}^2\text{)} = \text{Body weight (g)/Length}^2\text{ (cm}^2\text{)}$$

Sample collection

After recording the anthropometric measurements, orbital blood samples were obtained from the retro-orbital venous plexus using microcapillaries. The blood samples were collected in a clean, dry centrifuge tubes and allowed to clot to obtain sera. Serum samples were separated by centrifugation at 1800 ×*g* for 10 minutes at 4°C. Aliquots of serum samples were frozen and stored at −20°C pending further analysis. Following blood collections, animals were sacrificed by cervical dislocation, and a midline abdominal incision was performed, and whole liver of each animal was rapidly dissected out, thoroughly washed with ice-cold isotonic saline, blotted dry and then weighed. Each liver was divided into two portions. One portion was immediately homogenized to give 10% (w/v) homogenate in ice-cold medium containing phosphate buffer (pH: 7.4). The homogenate was centrifuged at 1800 ×*g* for 10 minutes at 4°C. The supernatant (10%) was separated and stored at −20°C for the determination of malondialdehyde (MDA) and nitric oxide (NO) contents. The second portion of the liver was fixed in 10% formalin saline for histopathological investigation.

Biochemical determinations

Serum total cholesterol, triglycerides, high-density lipoprotein (HDL), and serum glucose levels were assayed by colorimetric methods using Reactivos GPL kits (Barcelona, Espana) according to Meiattini [16], Bucolo and David [17], Naito [18], and Trinder [19], respectively. Serum low-density lipoprotein (LDL) level was quantified by colorimetric method using Centronic (Gmbh) kit (Wartenberg, Germany) according to Wieland and Seidel [20]. Serum insulin was estimated by enzyme-linked immunosorbent assay using Immunospec Corporation kit (USA) according to the method of Eastham [21]. Hepatic MDA content was determined by a colorimetric method using Biodiagnostic kit (Egypt) following the method of Satoh [22]. Hepatic NO content was estimated by colorimetric method using Biodiagnostic kit (Egypt) according to the method of Montgoery and Dymock [23]. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were determined by the colorimetric method using Salucea kit (The Netherlands) according to the method described by Young [24]. Serum total bilirubin was estimated by a colorimetric method using Biodiagnostic kit (Egypt) according to the method described by Walterv and Gerade [25]. Hepatic total protein level was determined by the colorimetric method of Lowry *et al*. [26]. Assessment-insulin resistant (homeostatic model assessment-insulin resistance [HOMA-IR]) was calculated using the following formula;

$$\text{HOMA-IR} = [\text{Fasting glucose (mg/dL)} \times \text{Fasting insulin (µIU/mL)}]/405$$

According to Matthews *et al*. [27].

Histopathological investigation

After fixation of liver specimens in formalin saline (10%) for 24 hrs, washing in tap water was done, and then the liver samples were subjected to serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) for dehydration. Afterward, liver specimens were cleared in xylene and embedded in paraffin wax at 56° in a hot air oven for 24 hrs. Paraffin wax tissue blocks were submitted for sectioning at 4 µm by Sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin [28]. After staining, the slides were viewed with an Olympus CH (Japan) light microscope [29].

Statistical analysis

In the present study, all results were expressed as mean±standard error of the mean. Statistical Package for the Social Sciences program, version 14.0 was used to compare significance between each two groups. Difference was considered statistically significant when $p<0.05$. Percentage difference representing the percent of variation with

respect to corresponding control group was calculated according to the following formula:

% Difference = (Treated value − control value)/control value) × 100

RESULTS

Anthropometrical measurements

The present study showed a significant increment (p<0.05) in TC (31.97%), AC (27.18%), and BMI (38.03%) of obese group with respect to control group. Meanwhile, obese group treated with ethanolic extract of *M. alba* or simvastatin led to significant reduction (p<0.05) in TC (−16.96% and −19.13%, respectively), AC (−18.40% and −19.83%, respectively), and BMI (−23.78% and −24.59%, respectively) as compared to obese group (Table 1). Interestingly, no significant alterations were observed (p>0.05) between the obese group treated with ethanolic extract of *M. alba* and that received simvastatin as for the anthropometric estimations (Table 1).

Biochemical determinations

Data in Table 2 illustrated the effect of ethanolic extract of *M. alba* administration on lipid profile of obese rats. A significant increase (p<0.05) in serum cholesterol, LDL, and triglycerides levels (104.18%, 90.16%, and 37.29% individually) was detected in obese group versus control group. Meanwhile, serum HDL level recorded marked reduction (p<0.05) in obese group (−47.24%) versus control group. On the other hand, obese group treated with ethanolic extract of *M. alba* or simvastatin exhibited a significant reduction (p<0.05) in serum cholesterol (−28.65% and −38.81% separately), LDL (−24.76% and −38.05% individually), and triglycerides (−21.18% and −32.53% individually) levels versus obese group. In contrast, a significant elevation in serum HDL level was demonstrated (p<0.05) in obese group received *M. alba* ethanolic extract (59.77%) or simvastatin (67.19%). It is worth mentioning that obese group administered *M. alba* ethanolic extract indicated a significant increment (p<0.05) in serum cholesterol, LDL, and triglycerides levels versus obese group administered simvastatin. However, serum HDL level recorded no significant change (p>0.05) in obese group received *M. alba* ethanolic extract as compared to obese group received simvastatin (Table 2).

Data in Table 3 revealed that serum glucose, insulin levels, and insulin resistant value were significantly enhanced (p<0.05) in obese group (152.64%, 49.06%, and 275.21%, respectively) versus control group. However, *M. alba* ethanolic extract or simvastatin administration resulted in significant reduction (p<0.05) in serum glucose level (−51.80% and −53.32%, respectively), insulin (−11.00% and −18.90%, respectively), and insulin resistant (−57.05% and −62.11%, respectively) values versus obese group. Interestingly, no significant alteration was detected (p>0.05) in serum glucose, insulin levels, and insulin resistant value between *M. alba* ethanolic extract and simvastatin-treated obese groups (Table 3).

Our findings in Table 4 represent the effect of *M. alba* ethanolic extract treatment on hepatic pro-oxidants content of obese rats. Where hepatic MDA and NO contents displayed significant elevation (p<0.05) in obese group (476.05% and 672.37%, respectively) versus control group. However, *M. alba* ethanolic extract or simvastatin-treated groups showed significant attenuation (p<0.05) in hepatic MDA (−68.35% and −73.85%, respectively) and NO (−64.41% and −76.37%, respectively) versus obese group (Table 4). Noticeable, no marked change (p>0.05) in

Table 1: Effect of *M. alba* ethanolic extract on anthropometric estimations in obese rats

Groups	Parameters		
	TC (cm)	AC (cm)	BMI (g/cm^2)
Control group	12.20±0.35	15.34±0.18	0.710±0.021
Obese group (Ob)	16.10±0.27[a] (31.97%)	19.51±0.31[a] (27.18%)	0.980±0.015[a] (38.03%)
Ob+*M. alba*	13.37±0.26[b] (−16.96%)	15.92±0.26[b] (−18.40%)	0.747±0.021[b] (−23.78%)
Ob+Sim	13.02±0.23[b] (−19.13%)	15.64±0.32[b] (−19.83%)	0.739±0.019[b] (−24.59%)

Data were displayed as mean±SE (n=8). [a]Significant difference at P>0.05 as compared to control group, [b]Significant difference at P>0.05 as compared to obese group. *M. alba*: *Morus alba*, SE: Standard error

Table 2: Effect of *M. alba* ethanolic extract on lipid profile in obese rats

Groups	Parameters			
	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Control group	49.56±2.02	49.65±1.71	35.29±1.82	13.42±0.94
Obese group (Ob)	101.19±1.75[a] (104.18%)	79.18±2.32[a] (37.29%)	18.62±0.82[a] (−47.24%)	25.52±0.91[a] (90.16%)
Ob+ *M. alba*	72.20±2.81[bc] (−28.65%) (16.60%)	62.41±0.68[bc] (−21.18%) (16.83%)	29.75±0.79[b] (59.77%) (−4.43%)	19.20±0.33[bc] (−24.76%) (21.44%)
Ob+Sim	61.92±1.86[b] (−38.81%)	53.42±2.31[b] (−32.53%)	31.13±1.24[b] (67.19%)	15.81±0.84[b] (−38.05%)

Data were exemplified as mean±SE of (n=8). [a]Significant difference at P>0.05 as compared to control group, [b]Significant difference at P>0.05 as compared to obese group, [c]Significant difference at P>0.05 as compared to simvastatin group. SE: Standard error, *M. alba*: *Morus alba*

Table 3: Effect of *M. alba* ethanolic extract on serum glucose, insulin levels, and insulin resistant value in obese rats

Groups	Parameters		
	Glucose (mg/dL)	Insulin (µIU/mL)	Insulin resistant (mg/dL.µIU/mL)
Control group	40.01±0.899	12.21±0.39	1.21±0.06
Obese group (Ob)	101.08±1.01[a] (152.64%)	18.20±0.32[a] (49.06%)	4.54±0.05[a] (275.21%)
Ob+*M. alba*	48.72±1.70[b] (−51.80%)	16.22±0.19[b] (−11.00%)	1.95±0.02[b] (−57.05%)
Ob+Sim	47.18±2.43[b] (−53.32%)	14.76±0.32[b] (−18.90%)	1.72±0.04[b] (−62.11%)

Data were displayed as mean±SE (n=8). [a]Significant difference at P>0.05 as compared to control group, [b]Significant difference at P>0.05 as compared to obese group. *M. alba: Morus alba*, SE: Standard error

Table 4: Effect of *M. alba* ethanolic extract on hepatic MDA and NO contents in obese rats

Groups	Parameters	
	MDA (nmol/mg protein)	NO (µmol/mg protein)
Control group	17.62±2.35	5.61±0.28
Obese group (Ob)	101.50±9.14[a] (476.05%)	43.33±5.84[a] (672.37%)
Ob+*M. alba*	32.12±4.22[bc] (−68.35%) (21.02%)	15.42±2.13[bc] (−64.41%) (50.59%)
Ob+Sim	26.54±3.21[b] (−73.85%)	10.24±1.06[b] (−76.37%)

Data were displayed as mean±SE (n=8). [a]Significant difference at P>0.05 as compared to control group, [b]Significant difference at P>0.05 as compared to obese group, [c]Significant difference at P>0.05 as compared to simvastatin group. *M. alba: Morus alba*, SE: Standard error

hepatic MDA content between *M. alba* ethanolic extract and simvastatin-treated obese groups. However, hepatic NO content demonstrated significant elevation (p<0.05) in *M. alba* ethanolic extract-treated obese group as compared to simvastatin-treated obese group (Table 4).

Results in Table 5 demonstrated liver functions recorded in obese rats after *M. alba* ethanolic extract administration. Where serum ALT (47.62%), AST (51.34%) activities as well as serum bilirubin (277.68%) level were significantly increased (p<0.05) in obese rats versus control group. On the other hand, obese groups treated with *M. alba* ethanolic extract or simvastatin delivered marked reduction (p<0.05) serum ALT (−15.89% and −24.89%, respectively), AST (−27.14% and −31.51%, respectively) activities, and serum bilirubin (−68.56% and −71.87%, respectively) level as compared to obese group. Of note, *M. alba* ethanolic extract-treated obese group indicated significant upregulation (p<0.05) in serum ALT activity and bilirubin level when compared to simvastatin-treated obese group. Meanwhile, serum AST activity did not differ significantly (p>0.05) in obese group received *M. alba* ethanolic extract from obese group received simvastatin (Table 5).

Histopathological investigation

Fig. 1 illustrated photomicrograph of liver tissue section of rat in the control group showing no histopathological alteration observed in the central vein and surrounding hepatocytes. Fig. 2 represented photomicrograph of liver tissue section of rat in the obese group showing ballooning degeneration in the hepatocytes associated with inflammatory cells infiltration in the portal area. Fig. 3 illustrated photomicrograph of liver tissue section of rat in obese group treated with ethanolic extract of *M. alba* showing dilatation and congestion in the central vein. Fig. 4 represented photomicrograph of liver tissue section of rat in the obese group treated with simvastatin demonstrating that the central and portal veins were dilated and congested.

DISCUSSION

Obesity is a chronic inflammatory disease recognized by elevated body weight and deposition of adipose tissue with extravagant fat storage [30]. Bioactive components from nature were indicated by many studies to possess a potential useful effect in treating obesity [31,32].

The current study went for investigating the possible effect of *M. alba* ethanolic extract in mitigating obesity-related hepatic steatosis and metabolic disorder represented by dyslipidemia, hyperinsulinemia, and glycemic status in obese rats.

Regarding anthropometric parameters, the present data revealed marked increment in the TC as well as AC in the obese group. Furthermore, BMI was significantly elevated in obese group with respect to control group. These results are in harmony with the data reported by Novelli *et al*. [13] who found fat aggregation in the thoracic and abdominal locales after feeding rats with HCD. Thus, the recorded increment in body weight could be attributed to the accumulation of adipose tissue and excessive energy intake. BMI has been expressed to be a reliable indicator of body fat and obesity in rats [13]. As daily lipid intake, BMI and fat deposition are positively correlated [33].

Treating obese rats with *M. alba* ethanolic extract or simvastatin (antihypercholesterolemic drug) resulted in significant reduction in TC, AC, and BMI as compared to obese group. The recorded drop in these anthropometric parameters upon treatment with *M. alba* ethanolic extract could be explained by its ability to decrease body fat proportion in obese rats. This effect could be attributed to the capability of active ingredients of *M. alba,* namely, mulberroside A, 5,7,2V-trihydroxyflavanone-4V-O-h-dglucoside and albanols A and B to suppress sterol regulatory component binding protein 1 release with subsequent inhibition in lipid aggregation and lipogenic genes expression, leading to a reduction in body lipids [34]. Moreover, *M. alba* contains cyanidin-3-glucoside, cyanidin-3-rutinoside, and pelargonidin-3-glucoside which might be contributed to repress body weight gain [35]. Furthermore, *M. alba* has been found to ameliorate adipocytokine dysregulation and suppress macrophage infiltration, which are involved in the development of obesity [36]. These may be the conceivable mechanisms implicated in weight reduction, and BMI regulation observed after *M. alba* ethanolic extract administration in obese rats.

The recorded alleviation in the anthropometric parameters in simvastatin-treated obese group might be explained by alteration in

Table 5: Effect of *M. alba* ethanolic extract on liver functions in obese rats

Groups	Parameters		
	ALT (U/L)	AST (U/L)	Bilirubin (mg/dL)
Control group	125.82±5.52	320.81±8.21	1.12±0.05
Obese group (Ob)	185.74±7.26[a] (47.62%)	485.51±9.27[a] (51.34%)	4.23±0.60[a] (277.68%)
Ob+*M. alba*	156.21±3.48[b] (−15.89%) (11.98%)	353.75±6.54[b] (−27.14%) (5.99%)	1.33±0.07[b] (−68.56%) (11.76%)
Ob+Sim	139.50±4.11[b] (−24.89%)	332.53±5.68[b] (−31.51%)	1.19±0.05[b] (−71.87%)

Data were exemplified as mean±SE (n=8). [a]Significant difference at P>0.05 as compared to control group, [b]Significant difference at P>0.05 as compared to obese group. *M. alba: Morus alba*, SE: Standard error

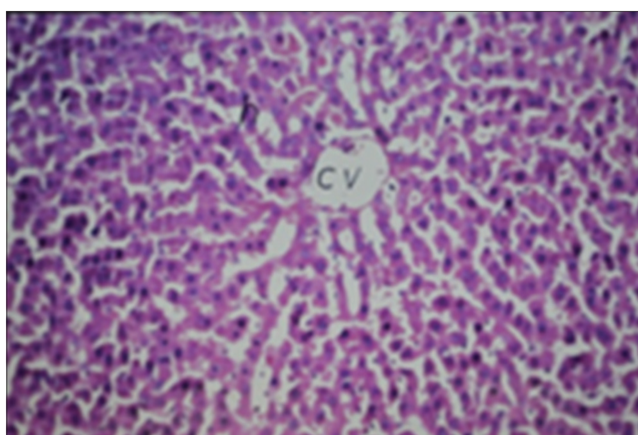

Fig. 1: Photomicrograph of liver tissue section of rat in the control group showing normal histological architecture of the central vein and surrounding hepatic cells (H and E, ×40)

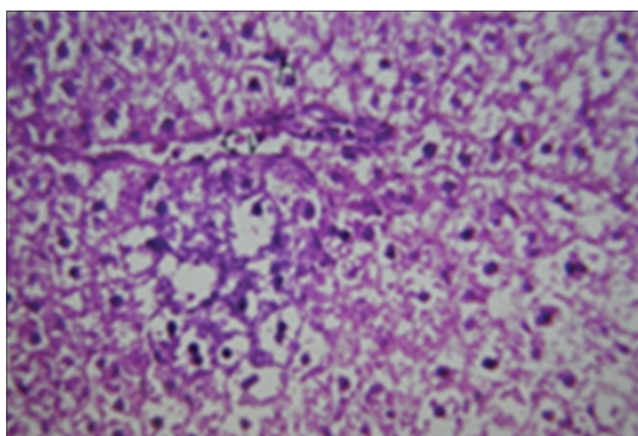

Fig. 2: Photomicrograph of obese group liver tissue showing ballooning degeneration in the hepatic cells and infiltrating inflammatory cells in the portal region (H and E, ×40)

adiponectin levels independent of adiposity induced by simvastatin supplementation [37]. Simvastatin affects atherogenic lipoproteins as well as reduces both LDL and triglyceride-rich lipoproteins, which forms non-HDL cholesterol. Moreover, a reduction in non-HDL cholesterol associated with an elevation in HDL cholesterol levels has been observed after administration of a higher dose of simvastatin [38].

The present results showed marked increment in serum cholesterol, triglycerides, and LDL levels associated with a significant drop in serum HDL level in obese rats regarding the control group. These data are in the same line with the previous findings of Son *et al.* [39] who reported significant elevation in cholesterol and triglycerides in obese rats. Furthermore, Fruchart *et al.* [40] stated that adipose tissue lipids are derived to a great extent from triglycerides particularly during elevated cholesterol diet intake. In addition, high cholesterol supplementation resulted in enhanced serum LDL level in obese rats [13]. This finding was clarified by the suppressed HDL level, as detected in our study, by suppressing the reverse cholesterol transport from circulatory system to the liver [41]. Moreover, elevated cholesterol diet causes oxidative stress prompting that generates reactive oxygen species (ROS), which causes cellular damage via oxidation of membrane lipids, proteins, and DNA of the cell. Thus, increased level of blood cholesterol particularly LDL is considered a major hazard for cholesterol-rich diet [42].

Most international and national lipid management protocols consider LDL cholesterol as a primary target of hypolipidemic therapy. *M. alba* ethanolic extract or simvastatin, used in the current study as therapeutic candidates could attenuate serum cholesterol, triglycerides, and LDL levels, whereas elevated serum HDL level relative to obese group. In accordance with our results, Lee *et al.* [43] indicated that *M. alba* could regulate energy balance and prevent metabolic disorders via regulation of lipid metabolism particularly triglycerides and cholesterol metabolism in obese mice. In addition, *M. alba* contains 24.3% dietary fiber, known to suppress triglycerides levels by inhibiting hepatic lipogenesis. It also reduces plasma LDL cholesterol by hindering the absorption of cholesterol and bile acid and enhancing LDL-receptor activity [44].

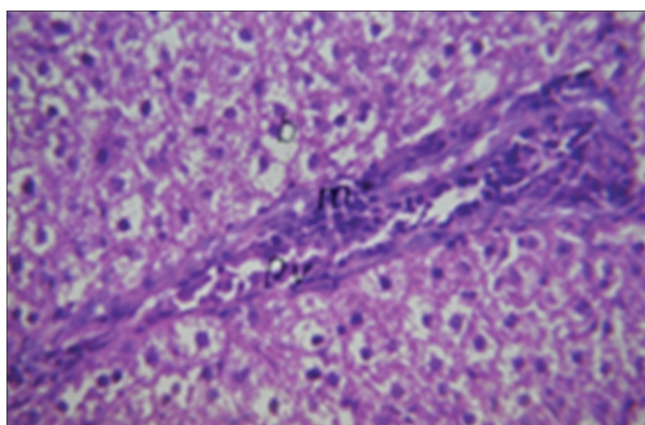

Fig. 3: Photomicrograph liver tissue of *M. alba* ethanolic extract-treated obese group showing dilatation and congestion in the central vein (H and E, ×40).

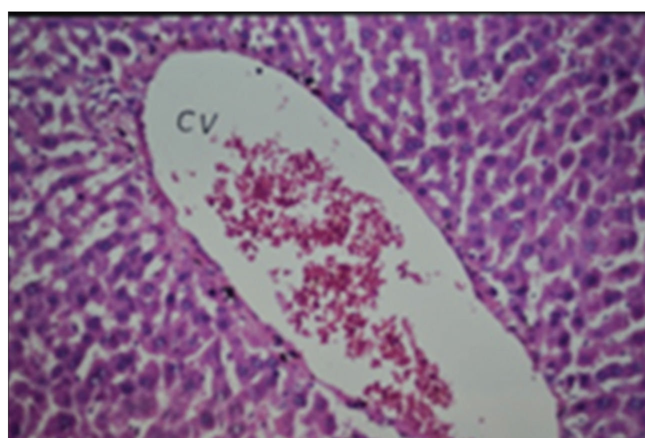

Fig. 4: Photomicrograph of liver tissue simvastatin-treated obese group showing that the central and portal veins were dilated and congested (H and E, ×40)

Treatment with simvastatin, in the present study, could ameliorate lipid profile by reducing serum cholesterol, triglycerides, and LDL levels and increasing HDL level versus obese group. Our results are in agreement with the previous reports [45,46]. Simvastatin belongs to inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a lipid lowering drug. Sabri *et al.* [47] demonstrated that simvastatin significantly reduced oxidative stress that contributes to obesity progression. It could significantly inhibit the induction of oxygen radicals subsequently, diminishes oxidative stress, increases HDL level, and improves lipid profile [48].

The present results demonstrated that obese group exhibited significant upregulation in serum glucose level relative to the control group. This result is in consonance with an earlier report of Galisteo *et al.* [49]. This finding could be attributed to enhanced lipolytic activity during fat aggregation causing high free unsaturated fats mobilization to the liver. Raised unsaturated fats flux to the liver enhances gluconeogenesis and diminishes the impact of insulin on peripheral glucose disposal [50].

The current results indicated that there was a significant decline in glucose serum level in the obese group treated with *M. alba* ethanolic extract versus obese group. Cyanidin-3-glucoside and cyanidin-3-rutinoside in *M. alba* could suppress body weight gain and body fat. Moreover, these active ingredients could decrease serum leptin levels and improve β cell function [35]. Furthermore, 1-deoxynojirimycin (DNJ), an active compound in mulberry leaves, has been reported to restrain intestinal α-glucosidases, which suppresses the absorption of glucose from the intestine [51]. More recently, it was found that the hybrid of DNJ and polysaccharide from mulberry leaves had antidiabetic effect *via* regulating the hepatic gluconeogenesis enzymes, glucokinase, phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase expression [52].

Supplementation of obese group with simvastatin resulted in significant reduction in serum glucose level relative to obese group. Simvastatin has been found to suppress elevation of intracellular Ca^{+2} level induced by glucose in a dose-dependent way. Furthermore, simvastatin could inhibit glucose-induced insulin secretion from islets [53].

Our results recorded a significant elevation in serum insulin level and insulin resistance in obese group versus control group. These findings come in accordance with those of Galisteo *et al.* [49]. This could be attributed to the correlation between obesity and chronic systemic inflammation which conceivably prompts insulin resistance [54].

Obese group supplemented with *M. alba* ethanolic extract showed a significant decline in serum insulin level and insulin resistance value as compared to obese group alone. These effects of *M. alba* extract may be due to the powerful anti-inflammatory action of its major constituents (prenylated flavonoid) [55]. This property inhibited pro-inflammatory cytokine production and enhanced anti-inflammatory mediators. Thus, *M. alba* ethanolic extract could suppress hyperinsulinemia in obese rats.

Results of the current study revealed a significant decrement in insulin level and insulin resistance value in the serum of simvastatin-treated obese rats as compared to obese group. Simvastatin possesses anti-inflammatory capacity as it could inhibit the expression of oxLDL-induced mRNA and the production of tumor necrosis factor-α (TNF-α)

and monocyte chemoattractant protein-1 [35]. In addition, simvastatin exerted anti-inflammatory action in adipose tissue via inhibition of endoplasmic reticulum stress. Suppression of endoplasmic reticulum stress in adipocytes represented an alternative mechanism of the pleiotropic action of statins.

The present study confirmed that lipid peroxidation, a downstream chain reaction initiated by free radicals, was activated as reflected by the increased level of lipid peroxidation product, MDA in the liver of obese group when compared with control group. This outcome is in conformity with that of Prasanna and Purnima [56]. Increased caloric intake results in a marked decline in mitochondrial fluidity and promotes ROS generation which in turn increases the hepatic content of MDA in obese rats [57]. Furthermore, the investigation of Novelli *et al.* [13] illustrated that BMI is positively correlated to lipid peroxides product concentration.

The significant decline in hepatic MDA content recorded in the obese group treated with *M. alba* extract versus obese group could be credited to the presence of rutin which is considered to be the most abundant phenolic compound in mulberry [58]. It is well known that phenolic compounds have powerful antioxidant activity. They have a strong ability to restrain lipid peroxidation via chain-breaking superoxide anion radical scavengers [59]. Thus, protecting LDL from oxidation [60].

The present results indicated significant suppression in hepatic MDA content in simvastatin-treated obese group in comparison with obese group. This effect could be due to its hypolipidemic effect and antioxidant potential [61]. Sabri *et al*. [47] showed that simvastatin could notably diminish oxidative stress, which is a contributory agent for obesity, by means of restraining the generation of oxygen radicals independent of its cholesterol lowering effects.

NO indicated significant elevation in liver tissues of obese groups versus the control group. Choi *et al*. [62] stated that NO generation increases in obese and not in healthy participants. NO is a free radical known to be included in the regulation of many physiological and pathophysiological procedures, for example, vasodilatation, energy balance, and inflammatory reactions [63,64]. NO synthase (NOS) regulates the synthesis of NO. NOS are three isoforms: Endothelial NOS (eNOS), neuronal NOS, and inducible NOS (iNOS) [63]. eNOS isoform promotes the generation NO in small amounts with vasodilatator and antiatherosclerotic capacity. In morbidly obese (MO) patients, the expression of eNOS is downregulated boosting endothelial dysfunction. iNOS expression is upregulated in MO patients by elevated concentration of tumor TNF-α and it induces vascular and pancreatic beta cells damage, enhancing obesity-associated metabolic disorder [63,64]. Chronic inflammation and oxidative stress represent key features of obesity and are strongly related to each other [65,66]. Inflammatory cytokines elevated in MO patients mediate upregulation of iNOs isoform which induces NO generation at 1000-folds more than eNOs. [67].

The present results demonstrated a significant decrease in NO content in the liver of obese rats received *M. alba* ethanolic extract versus obese group. This result is in keeping with the recent investigation of Eo *et al*. [68] who reported that *M. alba* could block NO production via suppressing iNOS overexpression. Furthermore, the previous study conducted by Choi and Hwang [69], revealed that methanolic extract from *M. alba* leaves and its subfractions (chloroform, butanol, and aqueous fractions) restrains NO generation and significantly declines TNF-α release in LPS activated RAW264.7 macrophages.

Supplementation of obese group with simvastatin resulted in significant reduction in NO content in the liver relative to obese group. Simvastatin was found to suppress iNOS expression and prevent the generation of excess NO and nitrosative tissue stress [70]. More recent study mentioned that simvastatin could block nuclear factor-κB signaling pathway activation which promotes TNF-α and iNOS genes transcription [71].

The significant increment in serum AST, ALT activity, and bilirubin level represents a clinical indicator for liver tissue damage caused by toxicants or disease conditions [72]. In obese group, the activity of liver enzymes (AST and ALT) in serum as well as serum bilirubin level was significantly elevated relative to those in the control group. The quantity of the released cellular enzymes detected in the blood indicates the change in plasma membrane integrity and/or hepatocytes permeability. Obesity is known to produce oxidative stress and promotes the generation of ROS, which leads to overproduction of peroxidized lipid molecule in liver tissue as indicated by enhanced MDA content in the liver. Overproduction of lipid peroxides causes destabilization in cellular lipid substances inducing oxidative damage, especially of membrane structures. This leads to the leakage of liver enzymes into the circulation. Moreover, lipid peroxidation products and NO overproduction can trigger the excitotoxic process causing an imbalance in cellular functions as indicated by the increased serum level of bilirubin in obese rats [57].

M. alba ethanolic extract-treated obese group exhibited marked improvement in liver functions as evident from the significantly blunted activity of liver enzymes in serum and the significant drop of serum bilirubin level in the treated rats relative to obese counterparts. The therapeutic effects of most medicinal plants are attributed to their antioxidant properties which in turn, could be ascribed to their antioxidant phytochemicals. The hepatoprotective effect of *M. alba* extract could be mainly due to its antioxidant and free radical scavenging properties of its active constituents which have been demonstrated in various studies [73]. Thus, this effect of *M. alba* extract confirms the ability of its active ingredients to maintain the architectural integrity of hepatocytes and in turn, restrict the leakage of liver enzymes into circulation. This indicates the membrane-stabilizing property of *M. alba* extract.

Administration of simvastatin in obese rats markedly reduced serum activity of liver enzymes as well as the serum level of bilirubin versus obese rats. Abbas and Sakr [74] reported that the protective effect of simvastatin on the liver was established by the significant reduction of oxidative stress and alleviation of liver functions. These findings are in accordance with the reported results of Cui *et al*. [75] who illustrated downregulation of liver enzymes in the serum of HFD-fed rats treated with simvastatin.

In the present study, the histopathological investigation of liver tissue specimens in obese rats showed ballooning degeneration (cytoplasmic vacuolization) in the hepatocytes (hepatic steatosis) linked with inflammatory cells infiltration in the portal zone. These findings are in agreement with those of Abbas and Sakr [74] who reported that the detection of oxidative damage in the liver of rats kept on high-fat diet for 15 weeks was indicated by changes in oxidative stress markers as well as by histopathological investigation that revealed cytoplasmic vacuolization, degeneration, necrosis, and fatty alteration of hepatocytes which confirmed that the cells were severely injured.

Here, in the favorable impact of the treatment with *M. alba* ethanolic extract on liver tissue was manifested by the remarkable amendment in the cell integrity as showed in the histopathological examination. This indicates that *M. alba* ethanolic extract could restore the structural organization of the liver. This effect could be attributed to the antioxidant effect of the mulberry constituents [34]. The leaf of mulberry contains triterpenes (lupeol), sterols (β-sitosterol), bioflavonoids (rutin, moracetin, quercetin-3-triglucoside, and isoquercitrin), coumarins, volatile oil, alkaloids, amino acids, and organic acids [76], which are considered to be valuable in inhibiting inflammatory and oxidative stress. Therefore, *M. alba* is considered as a major protector for cells against oxidative stress [77].

Administration of simvastatin in obese rats significantly improves hepatocyte degeneration as showed in the histopathological finding. This result is in line with the previously reported finding of Abbas and

Sakr [74] who demonstrated the improvement of liver tissue structure upon treatment of rats with simvastatin. This event could be explained by the hepatoprotective and antioxidant potential of simvastatin Cui *et al*. [75].

CONCLUSION

From the present experimental setting, it seems that successful obesity treatment will likely require intervention therapy that targets multiple systems. *M. alba* ethanolic extract imparted hypolipidemic effect, anti-inflammatory action, and antioxidative activity in mitigating obesity-induced hepatic steatosis and related aspects of metabolic disorder in the experimental model of obesity.

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